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Isolation and Identification of *Salmonella typhimurium* in local and imported frozen chicken meat in Province of Basrah/Iraq

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Abstract:

During the period from January to June 2023, 142 samples of local and imported frozen chicken meat were collected from Al-Basrah markets and surveyed for the occurrence of *Salmonella typhimurium*, depending on the area of collection, 72 samples from local frozen chicken and 70 imported frozen chicken. The number of samples that gave a positive result for *Salmonella typhimurium* (96) and identification was done according to IS06579 rules. Also, morphological, cultural, microscopical characterization, and biochemical tests were studied.

All the isolates were subjected to API 20E to ensure the identification, and the antibiotic sensitivity was investigated for all isolates of *S. typhimurium*, which showed multiple resistance patterns.

Keywords: *Salmonella typhimurium*, frozen chicken meat, gram-negative bacteria.

Introduction

Salmonellosis is a type of bacterial food poisoning caused by the *Salmonella* spp. The infection of Salmonella was the common cause of human food poisoning which represent a major problem for health in industrialized countries and albeit with low lethality and are defined as a group of microbial or toxic gastrointestinal diseases result from consuming contaminated food with certain microbial agent or their toxin. (1). There are several transmission routes for *Salmonellosis* but the most of human infections are derived from consumption of contaminated food especially those of animal origin (2). A variety of food product especially poultry meat are the most important sources of human *Salmonella* infection such as *S. typhimurium*, *S. enteritidis* (3). *Salmonella* is not known to release toxins into the contaminated food in which they are multiplying but the ingested bacteria is responsible for the disease by multiplying (4) in the intestine of the host and invading the git (5).

Salmonella is a genus of rod-shaped gram-negative bacteria of the forming motile with peritrichous flagella, some biochemical tests can be used to identify *Salmonella* such as fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indol production of H_2S (6,7).

Classification of *Salmonella*

Domain: Bacteria
Kingdom: Pseudomonadati
Phylum: Pseudomonadota
Class: Gammaproteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: *Salmonella*
Species: *typhimurium* (8).

In European union (Eu) *Salmonella* is the first notification cause of microbial food born contamination (9).

Chicken meat and its products are very popular food throughout the world which regarded as a good and cheap source of protein which easily digested, on the other hand they classified the first and second in the food associated with *Salmonellosis* in most of the countries all over the world (9).

The aimed of this study was to determine the incidence of *Salmonella typhimurium* in meat of frozen chicken using standard methods for the identification purpose.

Material and Method

Sample preparation: A total of 142 Samples of local and imported frozen chicken were brought from local market of Basrah city. The frozen chicken samples were transferred to the laboratory in an ice box. Frozen samples were thawed below 5C° for overnight refrigeration, then each sample was a septically and carefully freed from its casing and cut in half one half put in sterile polyethylene bag, added to it 250 of buffered peptone-water and shacked for 2-min mixed thoroughly in sterile – motor.

Salmonella spp. Was detected using standard bacteriological method (ISO 6579) (6). At the first culture were grown in buffered peptone-water (nonselective medium) for 18 h. at 37 C° then after incubation period the cultures were transferred to selective liquid medium (for enrichment); RaPPaPort- Vassiliad is medium with soy and Muller- Kauffmann tetrathionate novobiocin broth. The culture was incubated at 41.5 C° for 3 h. and 37 C° for 24 h. respectively identification were done on two selective media.

Xylose, Lysin deoxycholate agar and brilliant green agar a loopful was streaked on plates of both media then incubated at 37 C° for 24 h. After incubation examination for the presence of *Salmonella* colonies. The colonies which appeared after incubation period were red with or without black centers were sub cultured on nutrient agar plate incubated for 24h at 37 C°. Red colonies subjected to biochemical confirmation test according to standard IS06579-2002, by using Triple-sugar iron agar, Urease production, Indol reaction, Vogas Proskauer reaction and B-galactosidase (ONPG) test (10).

Red colonies also subjected to API 20E techniques to ensure identification (11).

For ensure the identification we used AP120 E. Which is plastic strip holds twenty mini test chamber containing dehydrated media with chemically defined composition

ONPG:O-nitrophenyl-beta-D-galactopyranoside, ADH: decarboxylation of arginine, LDC: lysine decarboxylation, ODC: ornithine decarboxylation ,CU: citrate utilization, H2S: production of H2S,URE:urease,TDA:tryptophan deaminase, IND: Indol test, VP: vogas proskauer, GEL: gelatin liquification,70GLU: glucose fermentation, MAN: mannose fermentation, INO: inositol fermentation, SOR: sorbitol fermentation, RHA: ramanose fermentation, SAC: sucrose fermentation, MEL: melibiose fermentation ,AMY: amgdalin fermentation, ARA: arabinose fermentation.

Antibiotics Susceptibility test

The antibiotics susceptibility test was done by the agar disc diffusion method as described by Kirby and Bauer (12).

Pure Isolates of *Salmonella typhimurium* were tested for susceptibility on Muller-Hinton agar plates (Difco). The disc of antimicrobial agent was used for this purpose showed in table (1) . Overnight cultures grown on autoclaved Muller-Hinton broth (Himedia- India) For 18 h at 37 C°. About 100 µl of the inoculum was spread on Muller-Hinton agar, using L-shaped spreader, and antibiotic disc were placed on the plates using sterile forceps, incubate the plates at 37 C° for 24 h. after incubation.

Results

The result in table (2) are summarized total of 142 sample of local and imported frozen chicken from different sites in Basrah city. The local samples 72 (58.3%) were found to be contaminated with *Salmonella typhimurium* while 70 (77.1%) of imported samples were found to be contaminated with *S. typhimurium* . The highest rate of *S. typhimurium* was iosolated from imported samples. From local samples the result in table (2) showed that the highest rate of *S. typhimurium* was isolated from 5 mile area 90% followed Alashar 66.6% Aljamait 52% and Aljazair 41.6%.

Also table (2) showed the highest contamination for imported samples at Al-Jamiat 100%. Followed by Al-Ashar 94.7%, Al-Jazair 68% and the lowest rate in 5-mile 47% statistical analysis.

The Statistical Analysis System SAS 2012 program was used to effect of different factors in the present study parameters least significant difference-LSD and Ducan test was used to significant compare between means.

Antibiotics susceptibility profile of *Salmonella typhimurium* isolated

S. typhimurium isolated were resistance or senstive to commonly used antibiotics are show in figure (1) the results indicated that the 96 isolates of *S. typhimurium* were resistant to the ampicillin (100%) , pencillin (100%) , vancomycin (100%) , for

sterptomycin (70%), tetracyclin (80%) ,erythromycin (70%) while for ciprofoxacin (30%), amikacin (10%) , gentamycin (10%) , and neomycin (10%).

There were significant differences ($p<0.05$) of *S. typhimurium* isolates between different type of antibiotics.

Table (1): The antibiotic disc used in the present study.

Antibiotic	**Concentration**
Ampicillin	10 Mg
Amikacin	30 Mg
Ciprofloxacin	5 Mg
Erythromycin	15 Mg
Gentamycin	10 Mg
Neomycin	10 Mg
Penicillin	10 Mg
Streptomycin	10 Mg
Tetracyclin	30 Mg
Vancomycin	30 Mg

Table (2) Determination of the number and percentage of *Salmonella typhimurium* isolated from local and imported samples from different places of Basrah City

Place Collection Samples	**Local Frozen Chicken**			**Imported Frozen Chicken**		
	No. of Samples	**No. of Positive Samples**	**%**	**No. of Samples**	**No. of Positive Samples**	**%**
5 – mile	**10**	**9**	**90**	**17**	**8**	**47**
Aljamaiat	**17**	**9**	**52**	**15**	**15**	**100**
Aljazair	**24**	**10**	**41.6**	**19**	**13**	**68**
Alashar	**21**	**14**	**66.6**	**19**	**18**	**94.7**
Total	**72**	**42**	**58.3**	**70**	**54**	**77.1**

($P<0.05$)

Table 3. Comparison Between Local and Imported Frozen Chicken for Contamination with *Salmonella typhimurium* with Different Places.

Place	Mean ± SE %		LSD value
	Local	Imported	
5 Mile	19.04± 1.62	14.18±1.58	6.15 NS*
Al Jamiat	Ab	Ac	7.84 NS*
Al Jazair	21.43+ 3.07	27.78±1.96	6.25 NS*
Al Ashar	Ab	A ab	4.73 NS*
	23.81± 3.38	24.07±2.54	
	Ab	Ab	
	33.33± 2.85	33.33± 2.64	
	A a	A a	
LSD value	8.613*	8.094*	

*(P <0.05) * NS: Non Significant

Mean having with different big letters in same row and letters in same column differ significantly.

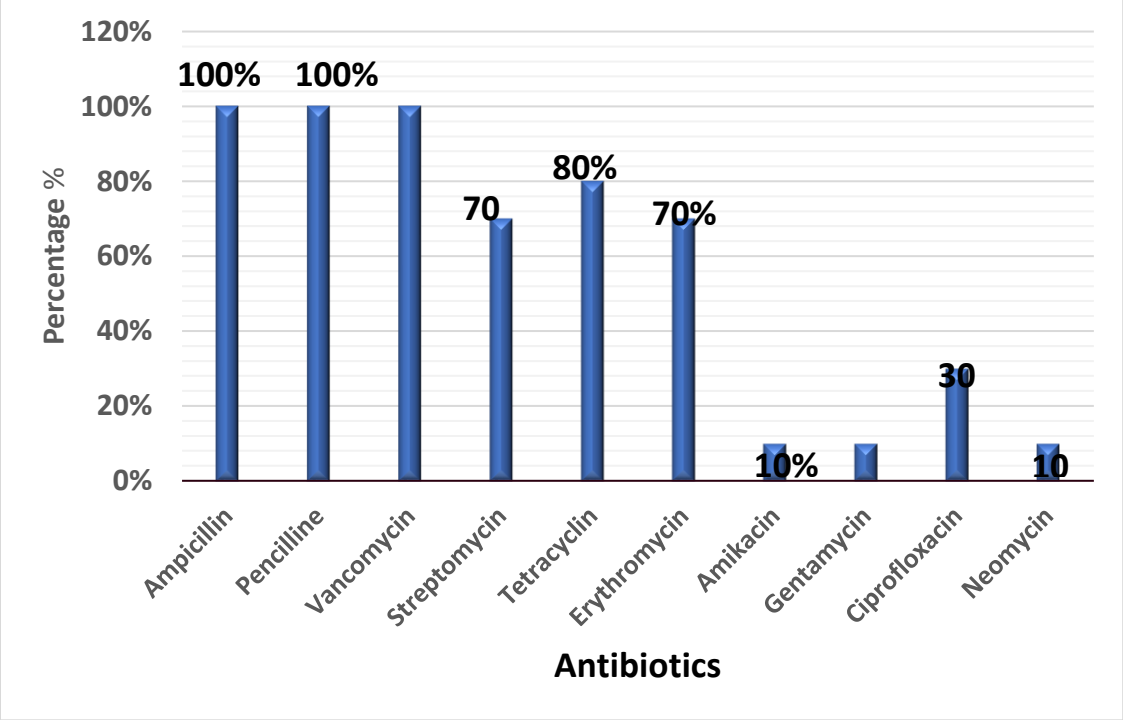

Figure (1): Antibiotic susceptibility profile of *Salmonella typhimurium* isolated.

Discussion

Salmonella typhimurium one of the most important bacteria isolated from food borne through out the world espacillay from raw chicken meat; the high incidence of *S. typhimurium* in food should be applied on prevention and control of contamination during processing for reducing food borne risks to consumers (13).Poultry meat commonly used for food especially chicken meats comprise about tow-thirds of the total production in the world (13). Chicken meats the most popular food products due to the nutritional properties such as high value of protein and toe fat content and good amount of unsaturated fatty acids. (14).

Chicken meats especialy frozen have always topped the incidence of salmonellosis in many countries such as Korea, India,Brazil (3), contamination occurred at multi-steps which starts from production , processing , distribution , retail marketing handling and preparation (15).

Therefore the detection of the prevalence of *S. typhimurium* in frozen chicken meat to take care during cooking and consumption of these foods.

In this study the present of *S.typhimurium* in frozen chicken of Basrah city were 58.3% for local samples and 77.1% for imported samples. These results agree with many studies in different region of the world (16), who isolated Salmonella of 27% in broiler chicken meat in Russian the results of this study revealed the presence of *S. typhmurium* in frozen chicken meat and this cross contamination and infection from foodborne Salmonella can caused to prolong illness and death in some cases. The data can helpful for good planning of food safety system for preservation and controlling of food against pathogenic bacteria. So this articles explained that the chicken meat is a vehicle of bacterial transfer agaents which play good role in disease prevalence and pathogen transmission to humen consumers especially for iraqi people the chicken meat regarded as a part of daily intake so this study may be one of important investigetion to indicate the contamination with this type of pathogen which showed the prevalence of varying drug resistance patterns (17). The antibiotic resistance can limited the therapeutic options available to physicians for clinical case using antimicrobial agents in food of animal like chicken for preservation caused gratly prevalence of antimicrobial resistant for different type of antibiotics, *Salmonella* is one of these organisims therefore there is a need to minimize the occurance of this pathogen in the food and study a stategies to decrease the risks of spreading antimicrobial resistance among human and animal population to reduce food poisining especially for chicken meat, good handling and hygienic food prepration, good storage and preservation all these can led to minimize the risks of food poisining with pathogens such as *Salmonella typhimurium.*

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عزل وتحديد السالمونيلا التيفوموريوم في لحوم الدجاج المجمدة المحلية والمستوردة في محافظة البصرة/العراق

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:ملخص

خلال الفترة من يناير إلى يونيو 2023، تم جمع 142 عينة من لحوم الدجاج المجمدة المحلية والمستوردة من أسواق البصرة وفحصها للكشف عن وجود سالمونيلا تيفيموريوم، اعتمادًا على منطقة الجمع، 72 عينة من الدجاج المجمدة المحلية و70 عينة من الدجاج المجمدة المستوردة. وقد أعطت 96 عينة نتيجة إيجابية لوجود السالمونيلا التيفوموريوم، وتم تحديدها وفقًا لقواعد .كما تمت دراسة الخصائص المورفولوجية والثقافية والمجهرية والاختبارات الكيميائية الحيوية .IS06579

لضمان تحديدها، وتم فحص حساسية المضادات الحيوية لجميع عينات API 20E خضعت جميع العينات المعزولة لاختبار .السالمونيلا التيفوموريوم، والتي أظهرت أنماط مقاومة متعددة

.الكلمات المفتاحية: السالمونيلا التيفوموريوم، لحم الدجاج المجمد، البكتيريا سالبة الجرام